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Expression of the type 1 human immunodeficiency virus Nef protein in T cells prevents antigen receptor-mediated induction of interleukin 2 mRNA

(signal transduction/Jurkat cells/interleukin 2 receptor α chain/calcium)

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ABSTRACT **Stable transformants of the Jurkat T-cell line have been obtained that express either of two distinct forms of the type 1 human immunodeficiency virus *nef* gene: the *nef-1*-encoded protein (Nef-1) contains alanine, glycine, and valine at positions 15, 29, and 33, respectively; the protein specified by *nef-2* (Nef-2) has threonine, arginine, and alanine at the corresponding positions. When Jurkat cells or their Nef-2-expressing transformants are treated with phorbol 12-myristate 13-acetate (PMA) plus either phytohemagglutinin (PHA) or antibodies against CD3ε, T-cell receptor β chain, or CD2, there is a prompt increase in interleukin 2 (IL-2) mRNA and intracellular calcium and in the IL-2 receptor α chain on the cell surface. Although cells expressing Nef-1 also induce calcium mobilization and the production of IL-2 receptor α chain, the formation of IL-2 mRNA is blocked in response to these stimuli. Moreover, Nef-1-expressing cells transfected with a plasmid in which the IL-2 promoter is fused to the chloramphenicol acetyltransferase (CAT) gene fail to induce CAT following treatment with PMA and PHA. By contrast, the parental and Nef-2-containing cells induce CAT normally. Nef-1-expressing cells can produce IL-2 mRNA in response to a combination of PMA and ionomycin, although much less efficiently than the parental Jurkat cells or Nef-2-expressing cells. These findings, and others described herein, suggest that the virally encoded Nef protein interferes with a signal emanating from the T-cell receptor complex that induces IL-2 gene transcription.**

Type 1 human immunodeficiency virus (HIV-1) contains seven genes (*tat*, *rev*, *tev*, *vpu*, *vpr*, *vif*, and *nef*) in addition to the signatory retroviral genes *gag*, *pol*, and *env*. Some of these genes have clearly established functions (1), whereas others are less well understood. The *nef* gene product (Nef protein), for example, is not required for HIV-1 multiplication in tissue culture cells (2, 3); indeed, viruses with defective *nef* genes replicate marginally more efficiently than the wild-type virus (3). Moreover, relative to their nontransformed parental cells, T cells stably transformed for *nef* expression exhibit a delay in viral multiplication following introduction of HIV-1 by infection or DNA transfection (4). Initially, Nef was thought to down-regulate transcription from the viral LTR (5, 6), but recent studies make this problematic (7–9).

Rather than concentrate on the role of Nef in the viral life cycle, we have investigated the effect of *nef* expression on host cell functions. The major cell targets for HIV-1 infection are $CD4^+$ T cells (10–12), most of which constitute the T-helper class (13). These $CD4^+$ T-helper cells bind antigen in the context of class II products of the major histocompatibility complex and consequently induce the transcriptional activation of a variety of lymphokine genes, including the interleukin 2 (IL-2) gene. These lymphokines are required both for the clonal expansion of the antigen-reactive T cell and for the efficient recruitment of other hemopoietic cells involved in the immune response.

Nef has been reported to bind GTP and to be a GTPase (14), properties it shares with the α subunits of trimeric G proteins and with Ras proteins, molecules which have known roles in signal transduction. Moreover, like members of the Src family, which are also involved in signal transduction, Nef is myristoylated at its N terminus and is associated with membranes (15, 16). These properties prompted us to determine whether Nef affects a T cell's ability to transduce the correct signal upon antigen binding. We reasoned that interference with signal transduction in HIV-1-infected T cells could potentially alter the levels of lymphokine production and might be expected to have profound effects upon the immune response.

Our results show that T cells constitutively expressing one form of Nef (Nef-1) are severely impaired in their ability to produce IL-2 mRNA in response to agents that mimic antigen binding to the antigen receptor. By contrast, cells that express Nef-2, which differs in three amino acids from Nef-1, induce IL-2 mRNA normally following the antigenic stimulus. Additional comparisons of the properties of Nef-1- and Nef-2-producing cells with their parental cells are also discussed.

MATERIALS AND METHODS

Construction of Nef Expression Plasmids. Full details of the construction of Nef expression plasmids, which were constructed by standard techniques (17), will be published elsewhere. A brief outline is provided in the legend to Fig. 1.

Preparation of Anti-Nef Antibodies. Polyclonal Nef antibodies were obtained from New Zealand White rabbits that had been injected with a TrpE–Nef fusion protein made in *Escherichia coli*. The *nef* gene from pNL432 (5) was amplified by the polymerase chain reaction using oligonucleotides spanning the coding sequence (21). The resulting *nef* DNA was cloned into pATH1 (22) to give a *trpE–nef* gene fusion. The TrpE–Nef fusion protein, produced after induction (22), was purified twice by PAGE and a gel slice containing the protein was used to immunize rabbits. After bleeding, the serum was used without further purification.

Cell Culture and Production of *nef* Transformants. The Jurkat human T-cell line J25 (obtained from G. Crabtree, Stanford University) was maintained in RPMI 1640 with 10% fetal bovine serum and penicillin (500 units/ml)/streptomy-

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Abbreviations: CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; IL-2, interleukin 2; IL-2Rα, IL-2 receptor α chain; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; TCR, T-cell receptor for antigen.

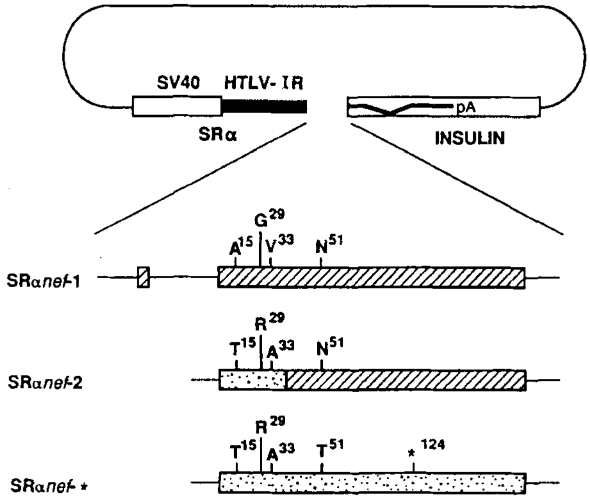

FIG. 1. Structure of Nef expression plasmids. The *nef* gene was expressed using SRα plasmids (18), whose promoter consists of a fragment of DNA from the R–U5 region of type I human T-lymphotropic virus (HTLV-IR, filled box) immediately downstream of the simian virus 40 (SV40) early promoter (open box). Sequences from the rat preproinsulin II gene (19) are 3′ to the *nef* gene to provide both an intron and a polyadenylylation (pA) signal. In SRαnef-1, sequences from the *Bam*HI site to the *Sac* I site of pNL432 (4) were used in the expression cassette. In SRαnef-2, sequences from the *Rsa* I site to the *Xho* I site of HXB2 (20) replace the sequence between the *Bam*HI site and the *Xho* I site of SRαnef-1. In SRαnef-*, the sequence between the *Bam*HI site and the *Sac* I site of SRαnef-1 was replaced by the sequence between the *Rsa* I site and the *Sac* I site of HXB2. SRαnef-1 and SRαnef-2 were used to generate stable Nef-expressing clones; SRαnef-*, which does not produce a stable Nef protein, was used in transient transfections as a control. DNA derived from pNL432 is shown by hatching, whereas DNA derived from HXB2 is shown by stippling. The coding potential of the plasmids was determined by dideoxy sequencing. Differences between the deduced amino acid sequences of the three plasmids as well as the location of the premature termination codon (*) in SRαnef-* are indicated, as is the presence of an upstream open reading frame with the potential to encode the tetrapeptide Met-Pro-Gln-Pro in SRαnef-1. The backbone of the SRαnef plasmids is from pBR322.

cin sulfate (100 μg/ml) in a 5% CO_2 atmosphere. Nef-expressing cell lines were isolated after electroporation (23) of J25 cells (1.6×10^7 cells in 0.8 ml) with 72 μg of Nef plasmid (Fig. 1) and 7 μg of either pSV2neo (24) or pSV2hph. The transfected cells were divided among 24-well dishes and grown for 40 hr before the addition of G418 (800 μg/ml) or hygromycin (50 μg/ml). In the latter selection, the hygromycin concentration was kept at 50 μg/ml for 1 week and then increased by 50 μg/ml every 2 days to a final concentration of 300 μg/ml. After about 3 weeks, cells from individual wells were screened for Nef expression by Western immunoblotting (25). Cells from positive wells were cloned, using a fluorescence-activated cell sorter, into 96-well dishes containing selective medium with 20% fetal bovine serum. Western blotting was used to identify Nef-expressing clones.

T-Cell Activation. Cells from exponentially growing cultures were activated by resuspension in fresh medium containing phorbol 12-myristate 13-acetate (PMA, 20 ng/ml; Sigma) and either phytohemagglutinin (PHA, 2 μg/ml; Sigma), anti-CD3ε antibody OKT3 (1 μg/ml; Becton Dickinson), anti-T-cell receptor (TCR) antibody C305 (10^{-4} dilution of ascites fluid provided by Arthur Weiss, University of California, San Francisco), anti-CD2 antibodies 101d2-4C1 and 1mono-2A6 (10^{-2} dilution of ascites fluid provided by Ellis Reinherz, Harvard Medical School), or ionomycin (2 μM; Calbiochem) and incubation at 37°C. The response to T-cell activation was monitored as indicated in the figure legends.

RESULTS

Identification of Nef-Expressing Cell Lines. Stable cell lines expressing Nef were identified by immunoblotting extracts obtained from cells harvested during logarithmic growth (Fig. 2). The mobility of the protein reacting with the anti-TrpE–Nef antiserum in the various clones is consistent with the 27-kDa size of Nef reported by others (4, 8, 9, 14, 16). Twelve independent cell lines expressing Nef were obtained; eight of these were derived from transfections with SRαnef-1, while the remaining four were from transfections with SRαnef-2 (Fig. 1). Several drug-resistant clones were also recovered that failed to express Nef (clones 10C3 and 6B6, Fig. 2); these cells also lacked *nef* DNA as judged by Southern blotting. Despite the fact that cotransfections were performed using a 10-fold excess of *nef* plasmid relative to *neo* plasmid, Nef expressing clones were rarer than expected. In fact, the majority of drug-resistant clones did not contain detectable Nef, and those that did contained one or at most three copies of *nef*. Perhaps high levels of Nef are cytotoxic.

Cells Expressing Nef-1 Fail to Produce IL-2 mRNA in Response to T-Cell Activation. The T-cell's antigen receptor (TCR) normally responds to ligand binding by inducing the synthesis of a variety of cytokines. Of the several cytokines that can be induced in the Jurkat T-cell line, the induction of IL-2 mRNA is the best characterized (26). Therefore, we compared cells expressing or not expressing Nef for their response to PMA and PHA, agents known to induce IL-2 production through the TCR pathway (27).

Because IL-2 mRNA production in Jurkat cells reaches a maximum ≈4 hr after the addition of PMA and PHA, that time was chosen to measure the response of the various Nef-expressing clones. Fig. 3 shows a representative Northern blot of the RNA obtained after treatment of three of the

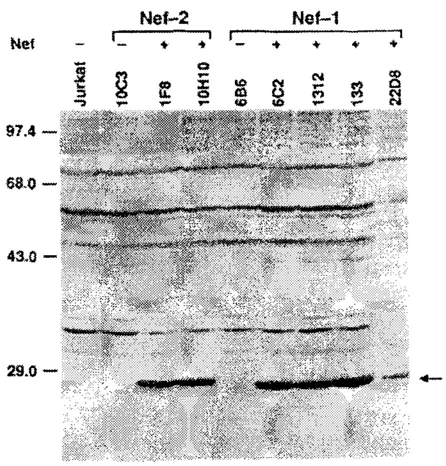

FIG. 2. Presence of Nef protein in stably transfected clones. Cells from logarithmically growing cultures were washed once in ice-cold phosphate-buffered saline, lysed in radioimmunoprecipitation assay (RIPA) buffer, and centrifuged at 98,000 rpm in a TA.100.1 rotor (Beckman) for 10 min. Lysates equivalent to 2×10^6 cells were then electrophoresed, the separated proteins were transferred to nitrocellulose, and Nef was stained by immunoblotting (25) using a biotinylated goat anti-rabbit IgG and a streptavidin-alkaline phosphatase kit (Vector Laboratories). Representative clones are indicated above each lane; Jurkat represents the parental cells; clones derived from transfection with SRαnef-1 or SRαnef-2 are bracketed; the presence (+) or absence (−) of Nef protein is indicated. Arrow at right indicates position of migration of Nef protein.

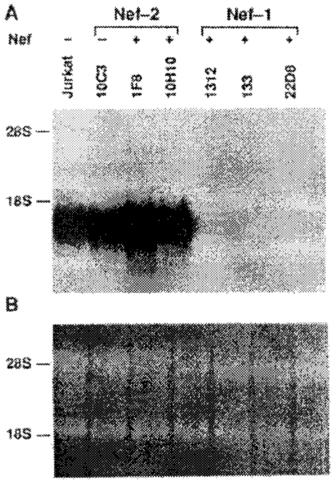

FIG. 3. IL-2 mRNA induction by Nef-expressing clones in response to PHA and PMA. RNA was prepared (28) from cells treated for 4 hr with PMA and PHA. IL-2 mRNA was detected by Northern blotting with a probe containing the 250-base-pair *Xba* I–*Hin*dIII fragment from pIL-2/12 (a gift from G. Crabtree) labeled to a specific activity of 5×10^8–2×10^9 cpm/μg (29). The source and designations of the RNA samples are the same as in Fig. 2. (*A*) Autoradiogram of the filter hybridized to the IL-2 probe: IL-2 mRNA migrates faster than the 18S rRNA. (*B*) Photograph of the ethidium bromide-stained gel from which the blot shown in *A* was obtained.

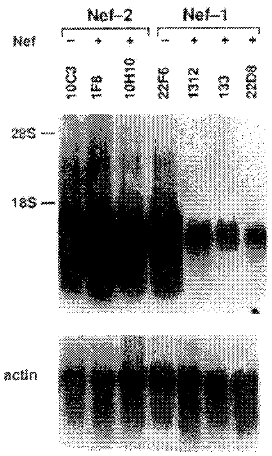

FIG. 4. IL-2 mRNA induction by treatment of stably transfected clones with ionomycin and PMA. RNA was prepared from cells after 4 hr of treatment with PMA and ionomycin and analyzed as described in Fig. 3. The annotations are the same as in Fig. 3. (*Upper*) Autoradiogram of the filter hybridized to the IL-2 probe. (*Lower*) Autoradiogram of the same filter rehybridized to an actin probe.

Nef-1 and two of the Nef-2 clones with PMA and PHA. Clones expressing Nef-1 were severely inhibited in their ability to accumulate IL-2 mRNA, whereas the Nef-2-expressing clones responded normally. This apparent failure of the Nef-1 clones to induce IL-2 mRNA was not due to an alteration in the kinetics of its accumulation, because IL-2 mRNA was not detectable between 5 min and 24 hr after induction. Furthermore, treatment with a combination of doses of PHA and PMA up to 5 μg/ml and 200 ng/ml, respectively, failed to induce IL-2 mRNA, indicating that the failure to respond was not due to an altered dose-responsiveness. Nef-1-expressing cells were equally unable to induce IL-2 mRNA when PMA was coupled with the more specific stimulation provided by monoclonal antibodies directed against either the TCR β chain, CD3ε, or CD2, whereas the Nef-2-expressing cells responded as well as the parental Jurkat cells. None of the clones synthesized IL-2 mRNA without induction.

Three Nef-1-expressing clones were exceptional. One clone, 14E6, which contains barely detectable levels of Nef as judged by immunoblotting, induces IL-2 mRNA normally after PMA and PHA treatment. Thus, the block in IL-2 induction may depend upon the level of Nef. Clone 5B2 expresses Nef-1 at a level equivalent to those shown in Fig. 2 yet it expresses IL-2 mRNA normally in response to induction. Further studies of this clone's properties are needed to resolve this exception. Clone 6C2, which expresses Nef-1, does not induce IL-2 mRNA. However, because 6C2 lacks surface CD3 expression (data not shown), its failure to induce IL-2 mRNA is not necessarily attributable to the presence of Nef-1.

Calcium Ionophore Partially Alleviates the Block to IL-2 mRNA Production in Nef-Expressing T Cells. Calcium ionophores can bypass the requirement for ligand binding to the TCR for IL-2 production (27). Consequently, we tested whether ionomycin in combination with PMA could induce IL-2 mRNA in Nef-expressing cells. Northern blots of the RNA obtained after induction of the Nef-expressing clones showed that all of the Nef-2-expressing clones, two of which are shown in Fig. 4, induced IL-2 mRNA as efficiently as clones not expressing Nef or the Jurkat parental cells. Five of the Nef-1-expressing clones, three of which are shown in Fig. 4, accumulated IL-2 mRNA in response to this treatment, albeit at reduced levels compared with the Jurkat or Nef-2-expressing cells. The three previously noted exceptional Nef-1-expressing clones (14E6, 5B2, and 6C2) responded normally to induction by the calcium ionophore.

Nef-Expressing Cells Increase Their Intracellular Calcium Concentration and Induce the IL-2 Receptor α Chain (IL-2Rα) Normally in Response to T-Cell Activation. T-cell activation induces several responses in addition to IL-2 synthesis. Among the responses are a transient increase in intracellular calcium (27) and the appearance of the IL-2Rα; the latter results in the formation of high-affinity IL-2 receptor (30). Nef-1- and Nef-2-expressing cells were indistinguishable from the parental Jurkat cells in their ability to mobilize intracellular calcium following treatment with either PHA or anti-CD3ε, as measured by flow cytometry of indo-1-loaded cells (31). No differences were detected either during the initial 10 min following stimulation or during 20-min intervals over the succeeding 90 min. Further, all of the Nef-expressing clones were indistinguishable from the parental cells in their ability to induce IL-2Rα. Whereas no IL-2Rα was present on the cell surface prior to the addition of PMA and PHA, flow cytometry showed that IL-2Rα on the cell surface increased following induction, reaching a maximum $\approx$24 hr postinduction (data not shown).

Nef-1 Blocks Transcription from the IL-2 Promoter in Induced T Cells. The ability of the Nef-1-expressing clones to induce calcium mobilization and IL-2Rα formation in response to TCR activation suggests that the cells are not in a generalized nonresponsive state. Therefore, we tested whether the block in IL-2 mRNA induction in Nef-1 expressing cells stems from the IL-2 gene promoter's inability to respond to TCR stimulation. For that purpose, Nef-expressing cells were compared with Jurkat cells for their ability to express chloramphenicol acetyltransferase (CAT) after transfection with a plasmid containing an IL-2 promoter–CAT gene fusion (32) and stimulation with PMA and PHA. CAT expression from the IL-2 promoter was markedly impaired in the Nef-1-expressing cells compared with Jurkat and Nef-2-expressing cells (Fig. 5, open bars). Similar indications that IL-2 promoter activation is impaired by Nef-1 were obtained when Jurkat cells were cotransfected with the IL-2–CAT gene and a *nef* plasmid and then induced with PMA and PHA (Fig. 5). SRαnef-1 caused a reproducible 50–60% inhibition of CAT expression compared with SRαnef-2 or the control plasmid SRαnef-*; essentially the same degree of inhibition of CAT expression was observed when the same cotransfections were made into Nef-2-expressing cells (Fig. 5). That CAT production is not more

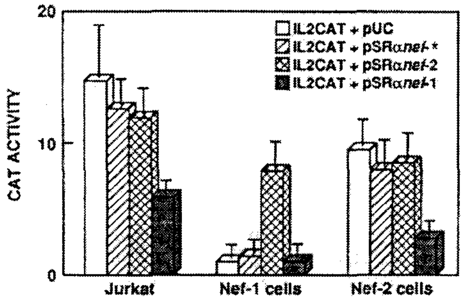

FIG. 5. Nef-1 impairs IL-2 promoter inducibility. Cells (10^7) from exponentially growing cultures were transfected by a modification of the DEAE-dextran method (33) with 22 μg of a 5:5:1 mixture of the indicated plasmid, the IL-2–CAT gene, and a Rous sarcoma virus promoter–luciferase gene construct. Forty hours after transfection, cultures were induced with PHA and PMA and after a further 12 hr cell extracts were prepared and assayed for CAT activity over an 18-hr incubation (34). CAT activities, expressed as percent conversion of chloramphenicol to its acetylated derivatives per 200 μg of protein after correction for luciferase activity, are the mean of at least three independent experiments; standard deviations are indicated by bars.

severely blocked by the cotransfected SRαnef-1 could be due to some cells receiving only the IL-2–CAT plasmid. SRαnef-* does not produce protein recognized by the anti-TrpE–Nef antiserum following transfection into COS cells (data not shown) and was therefore used to control for possible effects of SRαnef DNA or RNA sequences upon IL-2–CAT expression. Taken together, these experiments indicate that cells expressing Nef-1 are impaired in IL-2 promoter function in response to TCR activation.

Although Nef-2 fails to block IL-2 transcription after TCR activation, it can antagonize the inhibitory behavior of Nef-1. Thus, cotransfection of Nef-1-expressing cells with SRαnef-2 and the IL-2–CAT gene resulted in a nearly 10-fold induction of CAT formation following treatment with PMA and PHA (Fig. 5).

DISCUSSION

We explored the possibility that the HIV-1 *nef* gene product (Nef) affects an essential T-cell function rather than being needed for viral replication. To test that notion, *nef* was stably introduced into Jurkat T cells under the transcriptional control of a constitutive promoter. Two *nef* genes were examined: one (*nef-1*) encodes a protein with alanine, glycine, and valine at positions 15, 29, and 33, respectively, and the second (*nef-2*) differs only by encoding threonine, arginine, and alanine at the corresponding positions. Cells expressing either Nef-1 or Nef-2 were compared to the parental untransformed cells for their responses to agents known to mimic T-cell activation via the TCR.

Normal Jurkat cells and each of four independent clones expressing Nef-2 respond promptly to several stimuli known to induce IL-2 mRNA: PMA in combination either with PHA or with antibodies to CD3ε, TCR β chain, or CD2, or with ionomycin. By contrast, five of eight independent Nef-1-expressing clones appear to be unable to induce IL-2 mRNA after treatment with PMA in combination with any of the above mentioned external ligands. Because treatment of the unresponsive Nef-1-expressing clones with PMA and the calcium ionophore ionomycin results in a weak-to-moderate induction of IL-2 mRNA, we surmise that the IL-2 gene is still functional. Three of the eight Nef-1-expressing clones were exceptions. One (6C2) lacked CD3 and, therefore, its failure to induce IL-2 upon receptor activation cannot necessarily be attributed to the presence of Nef-1. Another (14E6) responds normally to receptor activation but it expresses considerably lower levels of Nef-1, suggesting that the amount of Nef-1 may be important for its inhibitory activity. The remaining exceptional clone (5B2) needs further study to explain its phenotype.

Mobilization of calcium and formation of the high-affinity IL-2 receptor are both well-documented additional inducible responses following TCR stimulation (27, 30). Yet, despite the failure of most Nef-1-expressing cells to produce IL-2 mRNA in response to TCR activation, the cells induce IL-2Rα and mobilize intracellular calcium normally. This indicates that only some of the signal-transduction pathways induced via the TCR complex are abolished or inhibited by Nef-1.

We surmise that the inability of Nef-1-expressing cells to induce IL-2 mRNA production by TCR activation stems from a block in the activation of the IL-2 gene promoter. This follows from our finding that an IL-2 promoter-driven CAT gene fails to be expressed after transfection into Nef-1-expressing cells following activation with PMA and PHA. By contrast, nearly equal levels of CAT are expressed in the parental Jurkat and Nef-2-expressing cells in comparable transfections. This result implicates the IL-2 gene promoter as a target of the Nef-1 inhibitory effect.

An important consideration relevant to our interpretation of the action of Nef is whether the cell's inability to induce IL-2 expression results from the presence of Nef *per se* or from some unknown property of the selected clones. The results obtained in experiments where the parental Jurkat cells were cotransfected with plasmids containing the IL-2–CAT gene and either of the two SRαnef genes, and then induced with PMA and PHA, clearly implicate Nef-1 as the activity responsible for blocking the induction of IL-2. Plasmids comparable to the SRαnef-1, in which the *nef-1* sequence is replaced by nef-2 or nef-*, have little or no effect on IL-2 induction. This finding indicates that Nef-1, but not Nef-2, prevents the induction of IL-2 following TCR activation. It will be particularly interesting to determine the structural basis for the functional difference between Nef-1 and Nef-2.

A further indication that Nef-1 is the activity responsible for the block to IL-2 induction comes from similar cotransfection experiments of the Nef-1-expressing cells. Here, cotransfection of IL-2–CAT and SRαnef-2 genes rescued the CAT inducibility of the Nef-1-expressing cells, whereas no such effect was observed when SRαnef-2 was replaced by SRαnef-1 or SRαnef-*. This indicates that Nef-2 can compete with Nef-1 for interaction with a critical component of the IL-2 induction pathway and that in doing so it relieves the inhibitory effect of Nef-1. Mutagenesis of Nef-2 should help define the regions important for this competition.

Previous studies have suggested that Nef acts to decrease viral multiplication (3–6). The most dramatic effect observed was obtained using T cells stably expressing Nef. Following infection with HIV-1 or transfection with a plasmid containing HIV-1 proviral DNA, cells expressing Nef were delayed in their production of virus relative to untransformed parental cells by at least 30 days (4). Other experiments analyzing the effect of *nef* upon viral multiplication have introduced mutant or wild-type forms of *nef* into cells during either infections or transient transfections (5–8). It is therefore unlikely that a stable, homogeneous level of Nef expression was obtained in the cell population being assayed. Consequently, we examined whether Nef affects transcription directed by the HIV long terminal repeat. This was done by transfecting the clones expressing Nef-1 or Nef-2 with an HIV CAT plasmid (35) and comparing their CAT production with the parental cells following stimulation with PHA and PMA, either in the presence or the absence of *tat*. The amount of CAT production was indistinguishable amongst the parental, Nef-1-, or

Nef-2-expressing cells under all the conditions tested (data not shown). This suggests that any affect of Nef upon viral multiplication occurs through a mechanism independent of transcription from the viral long terminal repeat.

How could Nef-1 prevent the induction of IL-2 following TCR activation? We assume, as have others, that antigen presentation to the TCR, or treatments mimicking that event, triggers an intracellular signal(s) that leads ultimately to the transcriptional activation of several genes, notably IL-2 as well as those encoding other cytokines (26). The nature of the molecules and reactions involved in that signal transduction is poorly understood, but activation of the TCR by treatments similar to the ones we have used has been found to induce a rapid conversion of Ras·GDP to Ras·GTP (36). Nef has limited but significant homology to the nucleotide-binding domains of Ras and Gα proteins and has been reported to bind GTP and to act as a GTPase (14). Although intracellular calcium is mobilized normally following TCR activation of Nef-1 cells, the block in IL-2 induction may nevertheless be influenced by the intracellular calcium level. This follows from our finding that the considerably greater rise in intracellular calcium produced by ionomycin is able to partially alleviate the Nef-1-mediated block of IL-2 production.

The phenotype of the Nef-1-expressing cells bears a striking similarity to the phenotypes observed in three recently described cases of severe combined immunodeficiency in human infants (37–40). In each of these cases, stimulation of the patient's peripheral blood lymphocytes with a variety of external ligands failed to induce IL-2 mRNA, despite being accompanied by the normal IL-2Rα induction and, where examined (37, 39), the normal rise in intracellular calcium concentration. Furthermore, the defect in these individuals may lie on the same pathway as that affected by Nef-1, since treatment of the cells with phorbol ester and calcium ionophore induced low levels of IL-2 mRNA in two of the cases (37, 40).

That cells expressing Nef-1 seem to be unable to produce IL-2 in response to TCR stimulation is of direct significance to the pathogenesis of AIDS. Because IL-2 binding to the high-affinity IL-2 receptor is necessary for cell division and therefore clonal expansion of the antigen-reactive cell, a T cell that fails to induce IL-2 in response to antigen is functionally absent from the individual's repertoire. But because Nef-expressing cells can induce IL2Rα, they can continue to divide as long as there is sufficient IL-2 made by cells whose signal-transduction system is functional. However, as the number of Nef-expressing cells increases, the amount of adventitious IL-2 available for clonal expansion of an antigen-stimulated Nef-containing cell would decrease, leading ultimately to the T-cell population's demise.

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